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Changes in pro-inflammatory cytokine levels and late-life depression: a two year population based longitudinal study

Running title: Cytokine changes and late-life depression

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Highlights

- A 2 year longitudinal study was conducted investigating cytokine levels and late-life depression.
- Depression at baseline was associated with higher contemporaneous cytokine levels.
- Incident depression was associated with increases in cytokine levels during follow-up.
- Incident depression was not predicted by cytokine levels at baseline.
- Depression may precede and lead inflammatory processes in late-life.
- Inferences can only be tentative, and future studies measuring central inflammation are needed

ABSTRACT

Longitudinal associations of cytokine levels with depression are unclear. This study aimed to investigate cross-sectional and prospective associations between five serum pro-inflammatory cytokine levels and late-life depression. 732 Korean people aged 65+ were evaluated at baseline. Of 631 without depression (Geriatric Mental State schedule) at baseline, 521 (83%) were followed over a 2 year period and incident depression was ascertained. Serum tumor necrosis factor-α, interleukin (IL)-1α, IL-1β, IL-6, and IL-8 levels were assayed at both baseline and follow-up. Associations between cytokine levels and depressive status were evaluated using linear regression models, considering potential covariates (demographics, cognitive function, disability, lifestyle factors, and vascular risk factors) and applying Bonferroni corrections. Prevalent depression at baseline was significantly associated with higher contemporaneous levels of IL-1β and IL-8, independent of relevant covariates and after applying Bonferroni corrections. In the analyses of the five cytokine levels in

combination, independent associations were found between prevalent depression and increased numbers of cytokines at higher levels at baseline. Incident depression was significantly associated with increases in IL-1β, IL-6, and IL-8 levels during the follow-up independent of relevant covariates and after applying Bonferroni corrections. In combination analyses, incident depression was independently associated with higher numbers of cytokines showing increasing levels over the same follow-up period. However, incident depression was not predicted by higher baseline pro-inflammatory cytokine levels in any analysis. Our findings suggest that depression might affect serum cytokines alterations and lead to inflammatory processes in late-life.

Key words: Depression, geriatric psychiatry, inflammation, cytokines, longitudinal studies

INTRODUCTION

Late-life depression is common and is associated with a substantial disease burden (Kok & Reynolds, 2017). With increasing life spans globally, the prevalence and morbidity of depression are expected to increase considerably (Kok & Reynolds, 2017). Understanding the etiology of depression in late life is an important step toward early detection and effective treatment. Late-life depression has complex and heterogeneous etiologies, including hypothalamic-pituitary-adrenal (HPA) axis dysfunction, vascular risk factors, and deficits in neurotransmitter signaling (Taylor et al., 2013). In addition, inflammatory processes have also been received attention (Leonard & Maes, 2012).

Cytokines are believed to play a pivotal role in the regulation of the inflammatory response. The involvement of pro-inflammatory cytokines (e.g., tumor necrosis factor alpha (TNF)-α, interleukin (IL)-1, IL-6, IL-8) in depression pathogenesis has been supported by animal and human studies (Dantzer et al., 2008; Maes, 2010). In animals, administration of pro-inflammatory cytokines has been found to induce depressive behavior (Dantzer, 2001), and some antidepressants have been suggested to have a cytokine-linked anti-inflammatory effect (Rana et al., 2016). In adult humans, a recent meta-analysis of 82 case-control studies reported that peripheral levels of cytokines including TNF-α and IL-6 were elevated in patients with major depressive disorder compared to healthy controls (Kohler et al., 2017). Similar findings have been reported for late-life depression from cross-sectional designs, including increased serum TNF-α and IL-6 associated with depression (Bremmer et al., 2008; Penninx et al., 2003; Tiemeier et al., 2003). Moreover, recent studies using [11C] PK11195 positron emission tomography, which can measure inflammation in the brain directly, suggested associations between neuroinflammation and depression both in adult and late-life

(Su et al., 2016; Holmes et al., 2017). However, causal relationships cannot be concluded from case-control and cross-sectional investigations, since altered cytokine levels may also be secondary to depression-related dysfunction (Kop & Gottdiener, 2005). Longitudinal studies maybe more appropriate for clarification, although these have been scarce and have reported inconsistent findings. The Sydney Memory and Aging study reported that two year incident depressive symptoms evaluated using the Geriatric Depression Scale (GDS) (Yesavage et al., 1982) were associated with IL-8 at baseline in elderly participants aged 70~90 years (Baune et al., 2012). The InCHIANTI (Invecchiare in Chianti, aging in the Chianti area) study reported that persons aged 65 or over with high IL-1 receptor antagonist level at baseline had a higher risk of developing depressive symptoms, evaluated using the Center for Epidemiological Studies–Depression Scale (Radloff, 1977) over a six year follow-up (Milaneschi et al., 2009). In both studies, incident depressive symptoms were not predicted by other serum cytokines including TNF-α and IL-6. Another longitudinal study in Italian residents aged 65 or over did not find any association between blood inflammatory proteins, including TNF-α or IL-6, and the 4-year risk of incident depressive symptoms evaluated using the GDS (Forti et al., 2010). Furthermore, the opposite direction of association has been reported, in that depressive symptoms evaluated using the Beck Depression Inventory-II (Beck et al., 1996) at baseline predicted changes in IL-6 levels, while baseline IL-6 levels did not predict depressive symptom changes in a six year longitudinal community study of healthy elders (Stewart et al., 2009). Summing up, the directionality of the inflammation and depression association has yet to be determined.

These discrepancies in previous findings might be due to the method employed for evaluating depression, in that heterogeneous assessment scales were used and case status defined on the basis of a cutoff score rather than a structured interview. In addition, most studies assayed

blood cytokine levels only at baseline, which is unlikely to determine the causal relationships. To address this, we analysed data from a two-year longitudinal study to investigate both cross-sectional and prospective associations of serum cytokine levels measured both at baseline and follow-up with depression in late-life, diagnosed by a widely used structured diagnostic interview.

METHOD

A secondary analysis was carried out on data from a community based prospective survey of late-life psychiatric morbidity carried out in Kwangju, South Korea from 2001 to 2003. All participants gave written formal informed consent at each examination. This study was approved by the Chonnam National University Hospital Institutional Review Board.

The baseline sample and measurements

A cross-sectional survey of a geographically defined population was carried out in 2001. The sampling procedure and measurements have been described previously (Kang et al., 2015). In brief, 732 community residents aged 65 or over within two defined geographic catchments of Kwangju, South Korea were recruited from national residents registration lists (5% refusal rate). Examinations included a fully structured diagnostic interview for depression; blood samples taken for five serum pro-inflammatory cytokines; and formal assessment of potential confounding factors.

Depression

Depression was assessed using the community version of the Geriatric Mental State schedule (GMS) (Copeland et al., 1986). The items for this instrument were derived mainly from DSM or ICD criteria with some modification for old age. The GMS is a fully structured diagnostic interview for mental disorders and focuses on the one month preceding the interview. It has been widely used in international epidemiological research for depression in late-life (Copeland et al, 1991), including research across European sites (Copeland et al., 1999), specific application and validation in East Asian communities (Kuh, 1992; Chong et al., 2001; Chen et al., 2004), and incorporation as the principal measure of affective disorder in the

10/66 research programme in Latin America, China and India (Llibre Rodriguez et al., 2008): the largest community study to date of mental health in older people. It takes approximately 30-40 minutes to administer by specifically trained interviewers. Diagnosis of depression in the last month is conventionally generated using the Automated Geriatric Examination for Computer Assisted Taxonomy (AGECAT) algorithm on a 0-5 scale. Participants rated as AGECAT 3, 4, and 5 are considered to be likely cases, those rated as 1 and 2 are considered to be sub-cases, and those rated as 0 to have no relevant symptomatology. With typical community prevalence estimates between 10-20%, the GMS-AGECAT depression criterion encompasses both moderate and severe symptomatology, and therefore a broader syndrome than DSM-IV major depression. Satisfactory agreement with Hamilton and Montgomery Asberg Depression scale cut-offs have also been demonstrated (Mottram et al., 2000). The GMS was translated into Korean according to a formal standardization process (Kim et al., 2003). As in other studies (Chen et al., 2004; Copeland et al., 1999; Llibre Rodriguez et al., 2008), a 'stage one' (non-hierarchical) confidence level of 3 or above from the AGECAT algorithm was used to define depression.

Blood samples and biochemical analyses

Participants were instructed to be fasting, and blood samples were carried out in the morning and were collected in a fasting state in 93% of participants. Venous blood samples were collected in tubes with no additives, and were centrifuged to isolate serum. Serum aliquots were stored at -70℃ within 2 hours of collection until analyses were performed. Biochemical assays were carried out for five serum pro-inflammatory cytokines: TNF-α, IL-1α, IL-1β, IL-6, and IL-8. Serum cytokine levels were measured using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen, Camarillo, CA, USA) according to the manufacturer's specifications. The intra-assay coefficients of variation were 3~6% for TNF-α,

4~6% for IL-1α, 4~7% for IL-1β, 4~5% for IL-6, and 2~4% for IL-8. The inter-assay coefficients of variation were 5~7% for TNF-α, 4~8% for IL-1α, 5~9% for IL-1β, 6~9% for IL-6, and 3~6% for IL-8.

Covariates

Factors associated with serum cytokine levels, and potentially confounding associations of interest, were investigated considering previous research findings (Baune et al., 2012; Milaneschi et al., 2009; Forti et al., 2010; Stewart et al., 2009). Age, gender, and education were recorded. Cognitive function was evaluated by the Korean version of the Mini-Mental State Examination (MMSE) (Folstein et al., 1975). Disability was assessed by the Korean version of the World Health Organization Disability Assessment Schedule II (WHODAS II) (Kim et al., 2005). Smoking history and current smoking status were ascertained. A lifetime history of alcohol consumption was obtained from the participants, and corroboration from family members was sought. Problem drinkers were defined on the basis of consumption over the previous three months of greater than 14 drinks per week for men or greater than 7 drinks per week for women, according to guidelines from the National Institute of Alcohol Abuse and Alcoholism (NIAAA, 1995). Self-rated physical activity was measured by asking about work and leisure activity over the previous month on the basis of a4-point scale (very active, fairly active, not very active, not at all active), and low physical activity was defined in case of not very active or not at all active as a binary variable according to the standard protocol adopted by the 10/66 Dementia Research Group (Prince et al., 2003). For vascular risk factors and disorders, self-reported disorders (stroke, heart disease, hypertension, diabetes), measured obesity (body mass index >25kg/m^2) and hypercholesterolemia (fasting cholesterol >200mg/dl) were evaluated. The presence or absence of any vascular risks was used in the analysis.

Follow-up evaluation

Follow-up was carried out in 2003 (Kim et al., 2008). The mean (SD) follow-up period was 2.4 (0.3) years. Attempts were made to follow up all previous participants. Identical procedures were used to identify depression (GMS-AGECAT) and further blood samples were collected, centrifuged within one hour and stored at minus 70°C. The same biomedical assays for the five serum pro-inflammatory cytokines were carried out using ELISA methods.

Statistical analysis

The study design for the present analysis is outlined in Figure 1. Cross-sectional associations between cytokine levels at baseline and depression at baseline were evaluated in all participants. Next, in order to analyse 'incident' depression, the sample was restricted to those without depression at baseline and comparisons made between those with and without depression at follow-up. Baseline demographic factors, assessment scales (MMSE and WHODAS II), lifestyle characteristics (smoking, problem drinking, and physical activity), and vascular risk score were compared between those with and without contemporaneous (baseline) depression using t- or χ^2 tests as appropriate. Those characteristics showing significant associations (p-value<0.05) were considered as covariates in the later multivariable analyses. Since the distributions of cytokine levels at baseline and at follow-up were positively skewed, these values were log transformed in the analyses. Initially, unadjusted associations of baseline serum cytokine levels with baseline depression were investigated using t-tests, as were associations of incident depression with previous baseline serum cytokine levels and with changes in levels from baseline to follow-up. Standardized β coefficients and standard errors were calculated for associations of baseline serum cytokine levels with baseline depression, of baseline serum cytokine levels with incident depression,

and of changes in serum cytokine levels (values at follow-up minus values at baseline) with incident depression; these were analysed both before and after adjustment for relevant baseline covariates using linear regression models. In addition, similar linear regression analyses were carried out in the total followed-up sample including those with baseline depression to evaluate associations of baseline cytokine levels with depression at follow-up, and of baseline depression with cytokine levels at follow-up. Since cytokines have previously been reported to have additive and synergistic effects on depression (Schiepers et al., 2005), combined effects of the pro-inflammatory cytokines were investigated as an exploratory procedure. Both baseline levels and changes in levels were categorised into lower / higher (below / above median value) binary variables, arbitrarily but in keeping with previous research (Bremmer et al., 2008), by summing up the number of higher cytokine levels at baseline, and the number of cytokine levels categorised as having greater increase over the follow-up period; these were then grouped into ordinal variables (0, 1, 2, 3, 4, 5) for each. Associations of baseline summed number of higher cytokine levels with prevalent depression, and of baseline and changed summed number of higher cytokine levels with incident depression were assessed initially by $\chi 2$-tests for linear trend (i.e. on one degree of freedom), and then by logistic regression models with adjustment for other covariates, reported as estimated odds ratios (95% confidence intervals) (ORs (95% CIs)). Bonferroni corrections were applied to maintain an overall type I error rate of 0.05 against the multiple comparisons. All statistical analyses were carried out using SPSS 21.0 software.

RESULTS

Participant recruitment and characteristics

The recruitment process and distribution of depression diagnoses are illustrated in Figure 1. Of 732 participants at baseline, case-level depression was present in 101 (13.8%). The severity of depression rated by AGECAT was that 460 participants were rated 0, 56 were 1, 115 were 2, 76 were 3, 13 were 4, and 7 were 5. Mean (SD) TNF-α, IL-1α,IL-1β, IL-6, and IL-8levels for the total sample were 43.4 (11.0), 12.4 (1.8), 9.9 (1.3), 21.9 (5.5), and 43.1 (9.7) pg/ml respectively. The cytokine levels were significantly correlated each other as shown in supplementary Table 1. Other characteristics of the sample and unadjusted associations with depression at baseline are summarized in supplementary Table 2. Depression was significantly associated with female sex, lower MMSE scores, higher WHODAS II scores, lower physical activity, and higher vascular risk score.

Of 631 participants without depression at baseline, 521 (83%) completed all evaluations at follow-up, incident depression was present in 63 (12.1%) of them. Baseline characteristics of participants at follow-up from the baseline non-depressed group are displayed in the last column of supplementary Table 2. Between the participants and non-participants at follow-up, there were no substantial differences in any independent variable (all p-values>0.06). Mean (SD) changes from baseline to follow-up in TNF-α, IL-1α,IL-1β, IL-6, and IL-8levels were +0.9 (9.4), +1.0 (1.7), +0.7 (2.8), +2.6 (8.1), and +1.3 (10.1) pg/ml, respectively.

Unadjusted associations of serum cytokine levels with depression status

Unadjusted associations of baseline and changes in serum cytokine levels with baseline and incident depression are summarised in Table 2. Prevalent depression at baseline was

associated with all five higher serum cytokine concentrations at baseline, and the strengths of association remained significant with higherIL-1β and IL-8 concentrations after applying Bonferroni corrections. Incident depression was associated with increased TNF-α, IL-1β, IL-6, and IL-8 concentrations during the follow-up period, and the strengths of association remained significant with increased IL-1β concentration after applying Bonferroni corrections. Incident depression was associated with higher IL-1α concentration at baseline, but the strength of association lost significance after applying Bonferroni corrections.

Adjusted associations of serum cytokine levels with depression status

Results of linear regression analyses are summarised in Table 3. Prevalent depression at baseline was significantly associated with higher IL-1β and IL-8 concentrations at baseline after adjustment for sex, cognitive function, disability, physical activity, and vascular risk score, and even after applying Bonferroni corrections. Incident depression was significantly associated with relative increases in IL-1β, IL-6, and IL-8 levels after adjustment, and even after applying Bonferroni corrections. Incident depression was not associated with any higher cytokine levels at baseline before or after adjustment, and after applying Bonferroni corrections.

Associations of serum cytokine levels with depression status in the total followed-up sample

Results of linear regression analyses in the total followed-up sample (N=610) are summarised in supplementary Tables 2 and 3. Five cytokine levels at baseline were not associated with depression at follow-up after adjustment for sex, cognitive function, disability, physical activity, vascular risk score, and baseline depression, and after applying Bonferroni corrections. However, prevalent depression at baseline was significantly associated with

higher IL-1β, IL-6, and IL-8 levels at follow-up after adjustment for sex, cognitive function, disability, physical activity, vascular risk score, and baseline corresponding cytokine levels, and even after applying Bonferroni corrections.

Summed measures of higher baseline cytokine levels and greater increase in cytokine levels by depression status

Combined associations of summed cytokine levels/changes with prevalent and incident depression are estimated. The number of baseline participants for each ordinal variable was 73 for 0, 143 for 1, 189 for 2, 124 for 3, 78 for 4, and 125 for 5; and that of followed-up participants was 31 for 0, 83 for 1, 228 for 2, 80 for 3, 81 for 4, and 56 for 5. In unadjusted analyses, prevalent depression at baseline was significantly associated with higher numbers of cytokines at high levels at baseline ($\chi2$=14.78; p-value<0.001). Incident depression was also significantly associated with higher numbers of cytokines showing increasing levels during follow-up ($\chi2$=79.91; p-value<0.001). No association was found between incident depression and the number of cytokines at higher levels at baseline ($\chi2$=0.544; p-value=0.461). Multivariable logistic regression analyses showed similar results. ORs (95% CIs) for the association between prevalent depression and increased summed number of higher cytokine levels at baseline was 1.26 (1.09-1.46), p-value=0.001; between incident depression and increased summed number of higher cytokine levels at baseline was 1.02 (0.86-1.21), p-value=0.791; and between incident depression and increased summed number of changed higher cytokine levels was 2.72 (2.13-3.49), p-value<0.001 after adjustment for sex, cognitive function, disability, physical activity, and vascular risk score.

DISCUSSION

Principal findings were as follows: i) prevalent late-life depression at baseline was associated with higher contemporaneous levels of IL-1β and IL-8 at baseline independent of relevant covariates and after applying Bonferroni corrections; ii) incident depression was significantly associated with increases since baseline in IL-1β, IL-6, and IL-8 levels after adjustment and after applying Bonferroni corrections; iii) prevalent depression at baseline was significantly associated with higher IL-1β,IL-6, and IL-8 levels at follow-up after adjustment and after applying Bonferroni corrections. In the analyses of the five cytokine levels in combination, independent cross-sectional associations at baseline were found between prevalent depression and number of cytokines at higher levels at baseline, and between incident depression and higher numbers of cytokine showing more pronounced increases during the follow-up. However, incident depression or depression at follow-up was not predicted by higher pro-inflammatory cytokine levels two years previously in any analysis.

A previous meta-analysis of 82 case-control studies using clinical samples reported significant associations between higher levels of pro-inflammatory cytokines and major depressive disorder in adults (Kohler et al., 2017). This finding has been replicated in cross-sectional studies with elderly populations (Bremmer et al., 2008; Penninx et al., 2003; Tiemeier et al., 2003). Our findings of cross-sectional associations between baseline depression and contemporaneous levels of the pro-inflammatory cytokines, IL-1β and IL-8 in particular, are thus consistent with those from previous studies. We also found that the five pro-inflammatory cytokine levels were significantly correlated with each other, as has been reported elsewhere (Bossa et al., 2014), and that baseline depression was independently associated with increased baseline number of pro-inflammatory cytokines at higher levels

(OR per unit increment = 1.26). Overall, therefore our cross-sectional findings suggest that higher serum pro-inflammatory cytokines are associated with depression in their combination as well as individually.

Longitudinal changes in pro-inflammatory cytokine concentrations around the onset of depression have received little evaluation. In our study, incident depression was strongly associated with parallel increases in cytokine levels from baseline to follow-up. The strength of this association was much greater than the contemporaneous cross-sectional one (OR per unit increment number pro-inflammatory cytokines showing a more pronounced increase in levels = 2.72). One possible explanation is that increases in pro-inflammatory cytokine levels might have depressogenic effects, consistent with the findings from animal studies on the pro-inflammatory cytokine induced depressive behaviours (Dantzer, 2001). However, the opposite causal direction is possible as well, as cytokine levels and depression status were evaluated only at baseline and at one follow-up point, and the relative timings of depression onset and cytokine increase were not ascertained. Therefore it is possible that depression related dysfunction could increase cytokine levels. Depression is closely associated with HPA axis hyperactivity and parasympathetic nervous system hypoactivity, both of which in turn can bring about inflammatory responses (Plotsky et al., 1998; Carney et al., 2005). In addition, a six year longitudinal community study with 263 healthy elders reported that depressive symptoms at baseline predicted changes in IL-6 levels, while baseline IL-6 levels did not predict depressive symptom changes (Stewart et al., 2009).

Previous investigations of baseline cytokine levels as predictors of incident depression have given rise to heterogeneous findings (Baune et al., 2012; Milaneschi et al., 2009; Forti et al., 2010; Stewart et al., 2009). These discrepancies might be due to differences in sample

characteristics, depression ascertainment, or blood assays. In our study, a higherIL-1α level at baseline was associated with incident depression in unadjusted analyses, but was no longer remained after adjustment. Moreover, no associations were significant when high cytokine categories were summed, unlike the other analyses described above. Summing up, our longitudinal observations lend more support to a hypothesis of cytokine alterations secondary to depression related dysfunction rather than depressogenic effects of higher cytokine levels. Our findings in the total followed-up sample of signification associations of prevalent depression at baseline with higher IL-1β, IL-6, and IL-8 levels at follow-up add support to the latter hypothesis. However inferences can only be tentative because of the uncertain temporal relationship described above between changes in cytokine levels and emergence of depressive symptoms; because of other potential confounders which could not be controlled; and because of potential "post-treatment" controls—e.g., vascular risk factors (recent smoking, drinking) at baseline could be sequelae of depression, and could lie on the causal path from that measure to cytokines. Also, inflammation may have a modulation effect on the progression of depression, thus playing a more complex role (Licinio & Wong, 1999). In addition, it is possible that the 2-year follow-up period was too long to detect depressogenic effects of high cytokine levels if these are short-latency.

Our study has several strengths in relation to previous research. First, depression was ascertained using a widely validated diagnostic schedule, rather than the brief assessment scales used in previous studies. Second, cytokine levels were measured both at baseline and follow-up points, which provided the opportunity to investigate changes in levels as a correlate. In addition, the follow-up rate was reasonable and not apparently differential with respect to risk factors of interest, and a large number of potential confounding factors were considered in the analyses.

Limitations of the study were that important other pro-inflammatory (e.g. IL-13, IL-18) and anti-inflammatory (e.g. IL-4, IL-10) cytokines were not measured; and that the incident depression outcome is unlikely to reflect fully complex symptom and syndrome trajectories as stated above. Duration of depression, important for the longitudinal analysis, could not be evaluated, since the GMS focuses only on the one month preceding the interview. In addition, the participants were recruited from a geographically defined rather than a nationally representative population, which may limit the generalizability of the findings. A further consideration is that the mean MMSE score of the participants was 23.3 (see Table 1), which is low for a typical high-income nation population. This is accounted for by the low educational level (mean 3.4 years) and high illiteracy (54%) in the sample, which can lower MMSE score particularly in calculation and language sections. However the findings from the regression analyses were robust after adjustment for the MMSE scores.

Our findings in this prospective community study suggest that depression may precede serum cytokines alterations and lead inflammatory processes, rather than that pro-inflammatory cytokines may have roles in the pathogenesis of depression in late-life. It has been reported that depression has adverse effects on both mental health including cognitive dysfunction and physical health including cardiovascular diseases (Ownby et al., 2006; Rozanski et al., 2005). The observed effects of depression could theoretically be mediated by inflammatory changes. Our findings need replication in future studies to confirm this hypothesis and with more diverse pro- and anti-inflammatory cytokines. In addition, genetic factors influencing cytokine levels and between-cytokine interactions require further evaluation, since there has been ample evidence that cytokine production is influenced by the transcriptional activity of

relevant polymorphisms (de Craen et al., 2005). Future studies measuring central inflammation are also needed, since central rather than peripheral inflammation may be more sensitive for late-life depression (Su et al., 2016).

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Conflict of interest

All authors declare that they have no conflict of interest.

Conflict of Interest

All authors reported no biomedical financial interests or potential conflicts of interest.

ETHICAL STATEMENT:

The study was performed in accordance with the Declaration of Helsinki.

The study was approved by the institutional review board of Chonnam National University

Hospital in Korea.

All procedures were carried out with the adequate understanding and written consent of the subjects.

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Figure 1. Flow chart of the study.

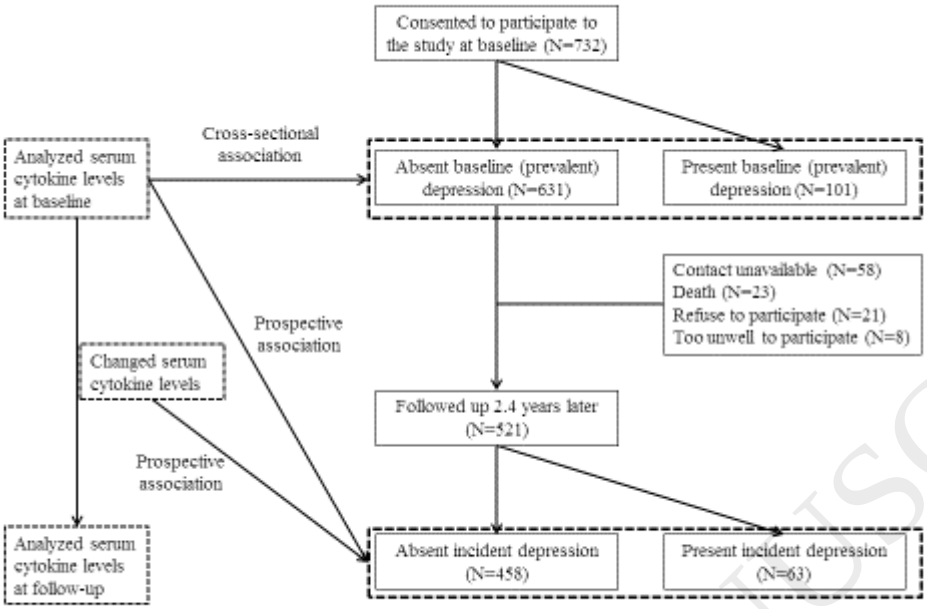

Table 1. Baseline characteristics by baseline depression status

	Participants at baseline				Analyzed participants at follow-up (N=521)
	Total sample (N=732)	No depression (N=631)	Depression (N=101)	P-value*	
Demographic characteristics					
Age, mean (SD) years	72.8 (5.9)	72.7 (5.8)	73.7 (6.3)	0.095	72.5 (5.5)
Female sex, N (%)	432 (59.0)	359 (56.9)	73 (72.3)	0.004	287 (55.1)
Education, mean (SD) years	3.4 (4.2)	3.5 (4.3)	3.1 (3.9)	0.406	3.5 (4.2)
Assessment scales					
MMSE, mean (SD) scores	23.3 (5.0)	23.5 (4.9)	22.1 (5.5)	0.006	23.7 (4.7)
WHODAS II, mean (SD) scores	7.1 (11.2)	5.9 (9.3)	15.1 (17.2)	<0.001	5.3 (8.6)
Lifestyle characteristics					
Current smoker, N (%)	294 (40.2)	250 (39.6)	44 (43.6)	0.453	209 (40.1)
Current problem drinker, N (%)	213 (29.1)	185 (29.3)	28 (27.7)	0.743	154 (29.6)
Low physical activity, N (%)	229 (31.3)	176 (27.9)	53 (52.5)	<0.001	139 (26.7)

Vascular risks, N (%)	476 (65)	397 (62.9)	79 (78.2)	0.003	321 (61.6)

MMSE: Mini-Mental State Examination; WHODAS II: World Health Organization Disability Assessment Scale II.

*t-test or χ^2 test as appropriate.

Table 2. Baseline levels of and follow-up changes in serum cytokine concentrations by baseline (prevalent) and incident depression status. Data are mean (SD)pg/ml.

Serum cytokine	Baseline (prevalent) depression			Incident depression		
	Absent (N=631)	Present (N=101)	P-value*	Absent (N=458)	Present (N=63)	P-value*
Baseline levels[a]						
Tumor necrosis factor- α	42.9 (10.8)	46.5 (11.6)	0.004	43.2 (9.6)	44.0 (11.1)	0.522
Interleukin-1α	12.3 (1.8)	12.8 (1.6)	0.015	12.2 (1.7)	12.8 (2.0)	0.021
Interleukin-1β	9.8 (1.3)	10.3 (1.3)	**<0.001**	9.8 (1.4)	9.9 (1.1)	0.495
Interleukin-6	21.6 (5.3)	23.4 (6.5)	0.009	21.5 (5.3)	22.5 (5.9)	0.162
Interleukin-8	42.6 (9.5)	46.2 (10.5)	**0.001**	41.9 (8.2)	42.9 (13.2)	0.567

Changes in levels over follow-up						
Tumor necrosis factor- α	-	-	-	+0.8 (8.0)	+3.2 (10.4)	0.032
Interleukin-1α	-	-	-	+1.0 (1.6)	+1.3 (2.1)	0.112
Interleukin-1β	-	-	-	+0.6 (3.0)	+1.7 (1.8)	**<0.001**
Interleukin-6	-	-	-	+2.4 (8.4)	+5.0 (6.8)	0.008
Interleukin-8	-	-	-	+1.0 (8.4)	+4.2 (15.5)	0.014

*t-test.

[a] Log transformed values were used in the analyses because the values were positively skewed.

Bold character denotes statistical significance after Bonferroni correction.

Table 3. Associations of baseline and changed cytokine levels with baseline (prevalent) and incident depression. Data are displayed as standardized β coefficients (standard errors).

Serum cytokine	Baseline (prevalent) depression		Incident depression	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
Baseline levels[b]				
Tumor necrosis factor- α	0.111 (0.001)†	0.102 (0.001)†	0.028 (0.003)	0.030 (0.003)
Interleukin-1α	0.089 (0.007)*	0.094 (0.007)†	0.114 (0.017)†	0.097 (0.017)*
Interleukin-1β	**0.136 (0.009)**‡	**0.113 (0.009)**†	0.030 (0.021)	0.003 (0.021)
Interleukin-6	**0.113 (0.002)**†	0.099 (0.002)†	0.062 (0.005)	0.053 (0.005)
Interleukin-8	**0.130 (0.001)**‡	**0.117 (0.001)**†	0.036 (0.003)	0.019 (0.003)
Changes in levels over follow-up				
Tumor necrosis factor- α	-	-	0.094 (0.003)*	0.087 (0.003)*
Interleukin-1α	-	-	0.072 (0.017)	0.090 (0.017)*
Interleukin-1β	-	-	**0.123 (0.010)**‡	**0.129 (0.010)**‡

Interleukin-6	-	-	**0.129 (0.003)‡**	**0.131 (0.003)‡**
Interleukin-8	-	-	0.108 (0.003)*	**0.115 (0.003)†**

[a] Adjusted for sex, cognitive function, disability, physical activity, and vascular risks.

[b] Log transformed values were used in the analyses because the values were positively skewed.

*p-value<0.05; † p-value<0.01; ‡ p-value<0.001.

Bold character denotes statistical significance after Bonferroni correction.